Commentary

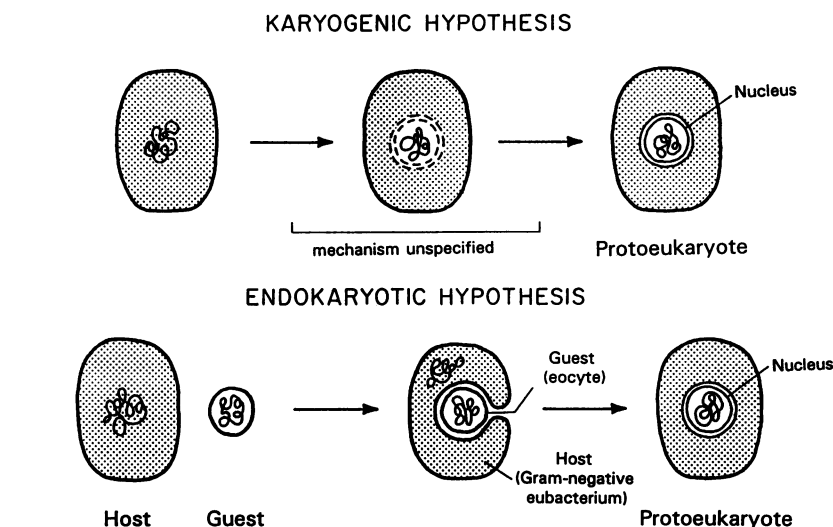# Was the nucleus the first endosymbiont?

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The evolutionary origins of the complex interconnected membrane system known as the endoplasmic reticulum have been enigmatic ever since its discovery and characterization by electron microscopy and biochemistry (1). These membranes are particularly important because, in addition to participating in protein segregation and secretion, they are continuous with the double-layer membranes that encircle the nucleus to form the nuclear envelope. Thus, they are thought to have evolved in concert with the nucleus.

Several theories exist for the origin of the nucleus. Many workers in the field subscribe to the *Karyogenic Hypothesis* illustrated in Fig. 1 *Upper* (adapted from ref. 2). In this theory the nucleus and its enclosing membranes were gradually acquired through some (unspecified) segregating process. The competing theory, although it has a rich history, is less well known. Quite early on (1900–1910), Pfeffer, Boveri, and Mereschowsky all considered a possible symbiotic origin for the nucleus (for a discussion and references, see ref. 3). It was not until electron microscopy established details of the organization of the nucleus that the *Endokaryotic Hypothesis* (4), illustrated in Fig. 1 *Lower*, was developed. This theory posits that the nucleus, like the other eukaryotic organelles enclosed in double membranes (the chloroplast and mitochondrion) has been derived through capture by an engulfing species. However, in this instance the guest (the nucleus), rather than being under the control of the host, has taken control of the host. The proposal is simple and parsimoniously explains the origin of all double-membrane organelles through a single mechanism rather than requiring two different mechanisms (one for the mitochondrion and chloroplast and another for the nucleus).

In this issue of the *Proceedings*, Gupta et al. (5) present data related to the origin of the endoplasmic reticulum and nucleus. They interpret their results as supporting the origin of the eukaryotic nucleus by an endosymbiotic event between two very different prokaryotes. One of these, the host, they believe to have arisen from within the Gram-negative bacteria and the other, the guest, they think is most likely an eocyte

KARYOGENIC HYPOTHESIS

mechanism unspecified   Protoeukaryote

ENDOKARYOTIC HYPOTHESIS

Host   Guest   Guest (eocyte)   Host (Gram-negative eubacterium)   Nucleus   Protoeukaryote

(a group of hyperthermophilic sulfur-metabolizing prokaryotes).

Gupta et al. base their interpretations on the sequences of the 70-kDa heat shock protein (HSP70). Two paralogous HSP70 genes are found in eukaryotes. One paralogue is present in the cytoplasm and the second, based on a characteristic N-terminal signal sequence and on a C-terminal endoplasmic retention sequence (6, 7), is present on the endoplasmic reticulum.

They chose to study *Giardia lamblia* because it lacks mitochondria and is thought to have branched from the eukaryotic evolutionary tree early in the formation of the eukaryotic lineage (refs. 8 and 9; see particularly the recent study by Hashimoto et al., ref. 10). Gupta et al. sequenced HSP70 from this early organism to determine if both the cytoplasmic and the ER forms were present. Based on their sequences and on the reconstruction of phylogenetic trees from eukaryotic sequences of both types, they concluded that both forms are present in *Giardia*. This suggests that the endoplasmic reticulum was already present at this early point in the evolution of eukaryotes.

To pursue the origins of the endoplasmic reticulum further, they also compared both HSP70 paralogues with the single copy that is present in prokaryotes. Both eukaryotic versions contain a 23-amino acid insert at approximately amino acid position 100. The insert is also

present in Gram-negative eubacteria but, unexpectedly, is absent in Gram-positive eubacteria, halobacteria, and methanogens. Hence, they reasoned that the ancestor of the eukaryotic HSP70s arose from within the eubacteria during a relatively late stage in their diversification. Their findings fit other observations that are emerging concerning the evolution of eukaryotic nuclear genes.

Eukaryotic nuclear sequences seem to be of two types. Some genes, especially metabolic housekeeping proteins, are closely related to their eubacterial counterparts. Horizontal gene transfers between prokaryotes and eukaryotes are suggested from analyses of the corresponding gene phylogenies. Examples of possible transfer from eukaryotes to eubacteria are glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate isomerase, and examples of possible transfer from eubacteria to eukaryotes include aldolase type II and Fe-containing superoxide dismutase (thoughtfully reviewed by Doolittle and co-workers, ref. 11). In contrast, other eukaryotic genes such as those encoding large- and small-subunit ribosomal RNAs, elongation factor EF-1α, and vacuolar-type ATPase sequences are more distantly related to their prokaryotic counterparts (25–50% amino acid identities for the proteins). Furthermore, these eukaryotic genes are more closely related to genes of

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the halobacteria, methanogens, and eocytes than they are to gene of the eubacteria (12, 13) and probably are most closely related to genes of the eocytes in particular (14–17).

The HSP70 proteins mimic the genes that are thought to be horizontally transferred from eubacteria to eukaryotes but differ in several ways. In particular, they are present in all eukaryotes so far studied, including the presumed earliest branch of eukaryotes and are present as paralogous copies in the cytoplasm and in the endoplasmic reticulum. This suggests that they entered the eukaryotic cell early in its history. Because of these characteristics and because both HSP70 paralogues share an insert with the Gram-negative eubacterium but not with the Gram-positive eubacteria, Gupta *et al.* propose that the original host may have been a Gram-negative eubacterium and that the original guest is likely to have been an eocyte. If this is the case, then one must still explain how the DNA of the original host cell was incorporated into the nucleus. This does not seem to raise much difficulty, however, given the numerous, well-documented examples in which chloroplast and mitochondrial DNA has been acquired by the nucleus (18, 19).

The choice of ancestors by Gupta *et al.* also may have some predictive power. For example, it may explain some irregularities in the distribution of ester lipids. It has long been puzzling that eubacteria, halobacteria, and eukaryotes share ester-linked fatty acids and functional fatty acid synthetases (20) and yet there is no phylogenetic indication that these three groups are closely related. However, if the fatty acids were introduced to eukaryotes through their host, and if that host was an eubacterium with ester lipids, then these data would most parsimoniously fit the eocyte phylogenetic tree.

Endosymbiotic hypotheses can be difficult to prove, and proving the *Endokaryotic Hypothesis*, involving events dating back to the beginnings of the eukaryotic cell, promises to be more difficult than proving the endosymbiotic origins of either the mitochondrion or the chloroplast. Nevertheless, Gupta *et al.* have made an important first step with their perceptive analyses of the HSP70 genes.

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